Egypt. Acad. J. Biolog. Sci., 5(2): 9-19 (2013) **G. Microbiology**
Email: egyptianacademic@yahoo.com **ISSN: 2090-0872**
Received: 25 / 4 / 2013 **www.eajbs.eg.net**

Probiotic stability of yoghurts during refrigerated storage

Ali, F. S.; Saad O. A.O and Salwa,A .G. Hussein
Department of Agricultural Microbiology, Faculty of Agriculture - Minia Univ. Minia Egypt.

ABSTRACT

Probiotic strains were isolated from the cheese, turkey and dimity and fermented products (rayb, zeer milk,kariesh cheese), screened for clotting time of skim milk. The seven effective isolates which clotting skim milk were identified as *Bifidobacterium spp* (RC1 b8, RC4 b2, FC1b1, SC1 a4, LZ1b8 and LZ1a7)and *Lactobacillus* spp (S4b1).

The seven effective strains of probiotic bacteria were used in the production of probiotic yoghurt 100%, yoghurt of 50% probiotic bacteria with 50% yoghurt bacteria and compared with the normal yoghurt with 100% normal yoghurt bacteria. The changes in pH, stability of bacteria, and physical properties in probiotic yoghurt 100%, yoghurt of 50% probiotic bacteria with 50% yoghurt bacteria and the normal yoghurt with 100% normal yoghurt bacteria during product's shelf life at 4C for four weaks were also studied.

Keywords: probiotic, yoghurt, antagonism and stability.

INTRODUCTION

Many probiotic bacteria used today in yoghurt grow poorly in milk when compared with some common starter cultures, such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Also, probiotic strains do not survive well in the final yoghurt product (Schillinger, 1999), or may even change the traditional taste of yoghurt. Although there are no set standards concerning the population of the probiotic organism in yoghurt at the end of the products shelf life, it is usually considered that anywhere between 5 and 8 log cfu g_1 is an acceptable final population (Shah *et al*, 1995; Schillinger, 1999; Svensson, 1999). Probiotics are live microorganisms which transit the gastrointestinal tract and in doing so benefit the health of the consumer (Tannock *et al.,* 2000). Therapeutic benefits have led to an increase in the incorporation of probiotic bacteria such as lactobacilli and bifidobacteria in dairy products, especially yoghurts (Lourens - Hattingh and Viljoen, 2001). There were significant strain differences in the viability of probiotic bacteria during storage of cultured dairy products (Ross *et al*., 2002).

Probiotics such as *Lactobacillus* and *Bifidobacterium spp*. are bacterial members of the normal human intestinal flora (Tamime *et al.,* 2005). Several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt and results in less than 108–109 cells daily recommended intake (Lourens-Hattingh and Viljoen, 2001). The suggested concentration for probiotic bacteria is in the range 106-107 cfu/g of the product (Robinson, 1987and Salminen *et al*.,1993).

Dairy products such as fermented milk and yoghurt are often used as carriers for probiotic cultures.

It was shown for the first time that probiotic and other lactic bacteria exhibit an antiviral activity in a cell culture model. Possible mechanisms of antiviral activity include: 1) hindering the adsorption and cell internalisation of the VSV due to the direct trapping of the virus by the bacteria, 2) "crosstalk" with

the cells in establishing the antiviral protection and 3) production of metabolites with a direct antiviral effect, Botić *et al.*,(2007).

High levels of viable microorganisms are recommended in probiotic foods for efficacy (Knorr, 1998).To achieve the claimed health benefits, one of the most important requirements for manufacturing and marketing of probiotic yoghurt is to maintain a high number of probiotic organisms P6 log CFU/g at the point of consumption (Lourens-Hattingh and Viljoen, 2001). Donkor *et al*., (2006) reviewed the factors that may affect the survival of *Lactobacillus* and *Bifidobacterium* spp. In yogurt: strain of probiotic bacteria, inoculation level, incubation temperature, pH, growth promoters and inhibitors, presence of hydrogen peroxide and oxygen, concentration of metabolites, lactic and acetic acid, buffering capacity of the media, storage temperature, and availability of nutrients. The main factors are ultimate pH and accumulation of organic acids (Dave and Shah, 1997and Shah, 2000).

Co-culturing with proteolytic yoghurt bacteria i.e. *Lactobacillus delbrueckii subsp. bulgaricus* (LB) and *Streptococcus thermophilus* (ST) also enhances the growth and viability of probiotics and helps to reduce fermentation time (Dave and Shah, 1997). To support the growth and survival of probiotic bacteria in fermented dairy products.

The aim of the present work to use the isolated probiotic bacteria which had strength of clot skim milk in the production of probiotic yogurt, Ali *et al.,(*2010 and 2012), to examine the changes in pH, stability of bacteria, and physical properties of seven probiotic strains in yoghurt alone or with 50% normal yoghurt bacteria compared with 100% normal yoghurt bacteria during product's shelf life at 4° C.

MATERALS AND METHODS

Yoghurt production:

For set-type yoghurt production, cows' milk was heated at 60–75C, homogenised under pressure of 100-200 bar, pasteurised at 85–95 °C and cooled at 37 to42 °C. After inoculation with the appropriate inoculum type, milk was distributed to 100mL plastic retail containers, sealed, incubated at 37 to 42 °C. until pH reached 4.6, and then cooled and stored at 4 °C. At time intervals of 1, 7, 14,21and 28 days, yoghurt samples were subjected to microbiological, and physicochemical analysis. All yoghurt trials were repeated in twice. Types of inocula and conditions used are shown in tables(1 to 4). When curdled milk inoculum was used, the milk was inoculated at 2% (v/v) with each strain and incubated at 37 or 42 °C. In each case the starter cultures were combined with one of the adjuncts. In addition, yoghurt was prepared with a 1% (v/v) inoculum and 37 to 42 °C incubation temperature, using the same starter cultures and 1%(v/v) traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus* and 2% (v/v) traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus.*

Assessment of yoghurt during refrigerated storage:

The inoculated milk samples were aseptically transferred into 100 ml plastic containers,tightly sealed, incubated at 40 °C overnight and transferred to fridge at 4 °C the following day. All trials were replicated three times. The sampling schedule for testing was immediately after the addition of starter culture into milk (day 0), after overnight incubation (day 1), fermentation and at weekly intervals (days 7, 14, 21 and 28). The analyses were performed on triplicate samples for pH, and in duplicate for

bacterial counts, firmness of yoghurt gel (curd tension)and syneresis.

Analytical determinations of set yoghurt:

Determination of pH:

The pH of yoghurt was measured using a bench-top pH-meter which was previously calibrated with pH 7.0 and 4.0 standard buffers.

Enumeration of probiotic and starter cultures:

Ten gram samples of each yoghurt batch were diluted with 90 ml of 0.1% sterile buffered peptone water (Oxoid Ltd.,Hampshire, UK) and placed in stomacher for 2 min. Tenfold serial dilutions of 10_2–10_9 were then prepared in 9 ml of 0.1% sterile peptone water and 1 ml of the three highest dilutions was pour-plated in duplicate. Colonies count of strains were enumerated on MRS agar under anaerobic condition at 30 ◌̊C for 72 h. The numbers of colony forming units (CFU) on plates containing 25–250 colonies were calculated per gram of sample.

Firmness of yoghurt gel (curd tension):

The firmness of the formed yoghurt gel was expressed as the weight (gm) required for a plastic tube to penetrate into the gel from surface to bottom.

Syneresis:

Amount of whey drained(ml/100gm of yoghurt within 30 minuts).

Statistical analysis:

All results were statistically analysed using Duncan's Multiple Range test (DMRT,1955) was applied for mean comparison when one-way analysis of variance (ANOVA) showed significant differences at the 95%confidence level.

RESULTS

a- Changes in pH during yoghurt storage:

Probiotic bacteria are slow acid producers (Marshall and Tamime, 1997). The yoghurt starter cultures including *L. delbrueckii subsp bulgaricus* and *S. thermophilus* are active even at refrigerated temperature and still can produce small amounts of lactic acid by fermentation of lactose which results is noticeable pH decrease (Shah *et al.,* 1995). Several reports have indicated that there are significant differences between probiotic strains with respect to survival in acid environment. The problem of sensitivity to acidity of the probiotic culture is compounded by the fact that acidity may increase during storage, a phenomenon known as 'over acidification'.

This post-acidification, during storage, is due to b-galactosidase which is still active at 0–5 ◌̊C. (Kailasapathy, 2006).

The pH in the control (Y.S.) and the experimental yoghurt (7 bacterial isolates, Fig.1, alone and mixed with Y.S. at 1:1) during storage at 4C for 4 weeks shown in Table (1). In fresh yoghurt, the control showed the lowest pH (3.4), followed by the treatments of the mixed isolates + Y.S., which exhibited slightly higher pH values (3.5 - 3.7). In case of the isolates alone, only 3 isolates exhibited pH values < 4.0 (3.6 – 3.9), while the rest isolates exhibited higher pH values (4.1 – 4.6). During storage, the pH in all treatment remained stable or slightly increased till the 4th week, except for the treatments of LZ1b8, RC1b8 and their mixture with Y.S. Generally, it was obvious that the pH values for the treatments of the individual isolates were always higher than for the treatments of the isolates mixed with Y.S.

The control yoghurt with the traditional yoghurt starter cultures showed the lowest pH. This finding may explain the result of high pH values for the yoghurts based on the individual isolates. Also, the yoghurt starter cultures including *L. delbrueckii var. bulgaricus* and *S. thermophilus* proved to be active even at refrigeration and can

produce small amount of lactic acid by fermentation of lactose which result in noticeable pH decrease (Shah *et al*., 1995). This statement also explains the lower pH values obtained in the control yoghurt and the treatments of mixed isolates with Y.S., where both contained *L. bulgaricus* and *S. thermophilus*. The initial pH prior to storage ranged from 4.2 to 4.5 that dropped to 4.1–4.3 after 28 days (Paseephol.and. Sherkat., 2009).

Table 1: Changes in pH of yoghurts based on individual probiotic isolates or mixed with traditional yoghurt starter (YS) during storage at 4 ◌̊C.

Treatment (Starter)	Source	pH of yoghurt stored at 4C.				
		24 hrs	1 week	2 weeks	3 weeks	4 weeks
Control	(Y.S.) (1)	3.4	3.4	3.9	3.9	3.8
RC1b8(2)	Ras cheese	4.6	4.4	5.3	4.9	4.4
RC1b8+Y.S.(3)		3.6	3.3	3.8	3.8	3.5
RC4b2	Ras cheese	4.1	4.1	4.4	4.4	4.7
RC4b2+ Y.S.		3.7	3.8	3.8	3.8	4.3
FC1b1	Domiatii cheese	3.9	3.9	3.9	3.9	4.4
FC1b1+ Y.S.		3.5	3.9	3.8	3.7	4.2
SC1a4	Karish cheese	4.6	5.1	4.7	4.8	5.0
SC1a4+ Y.S.		3.7	3.8	3.6	3.7	4.2
S4b1	Raybe milk	3.6	3.8	3.7	3.7	4.2
S4b1+ Y.S.		3.6	3.7	3.8	3.7	4.2
LZ1b8	Zeer milk	3.9	3.4	3.9	4.0	3.6
LZ1b8+ Y.S.		3.7	3.2	3.8	3.7	3.5
LZ1a7	Zeer milk	4.2	4.2	4.2	4.4	4.7
LZ1a7+ Y.S.		3.6	3.9	3.7	3.8	4.2

(1) Y.S. = Traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus.*

(2) Probiotic isolate alone as a starter (in skim milk).

(3) Probiotic isolate mixed with Y.S. at a ratio of 1:1.

B- Bacterial culture stability in yoghurt:

There are significant strain differences in the viability of probiotic bacteria during storage of cultured dairy products (Ross *et al*., 2002).The viability of 7 bacterial isolates(Ali *et al.,* 2012) either alone or in the presence of a traditional yoghurt starter (Y.S.) culture during storage of yoghurt at 4 ◌̊C for 4 weeks is presented in Table (2).

It is clear that the initial counts at zero time (immediately after inoculation) in all treatments were considerably higher (ranged 2.5x107 – 1.1x109 cfu /ml) than the control (Y.S. = 5.6x106 cfu/ml). The yoghurt kept in refrigerator overnight (approx. 24 hrs) exhibited a remarkable increase in the viable counts of all isolates alone and the control (almost 10-folds) as compared to the slight or no increase in the viable counts of bacterial mixtures (isolate +Y.S.). The presence of *L. delbrueckii bulgaricus* in Y.S. could be responsible for the reduced viable counts due to decreasing the pH (Table 1), in the mixtures treatments as compared to the yoghurt from the isolates alone. Rybaka and Kailasapathy (1995) reported that the presence of *L. bulgaricus* was found to be the main detrimental factor responsible for *Bifidobacterium sp.* mortality, and when *L. bulgaricus* was excluded from yoghurt manufacture, the decrease in pH was significantly reduced during storage, as *L. bulgaricus* cause over acidification during manufacture and storage. Also, Dave and Shah (1997) mentioned that the viability of *L. acidophilus* was badly affected by the presence of *L. delbrueckii bulgaricus*. Many studies have shown low viability of probiotics in yoghurt (Dave and Shah, 1997; Kailasapathy and Rybka, 1997; Shah, 2000 and Lourens-

Hattingh and Viljoen, 2001). The low viability in yoghurt is mainly attributed to the lower pH in yoghurt and further reduction of pH in yoghurt during post-acidification. During storage, the counts of the control remained almost stable till the second week, then increased remarkably (10-folds) in the 3rd week, followed by an obvious decline in the 4th week.

Treatment (Starter)	Source	pH of yoghurt stored at 4C.					
		Zero time	24 hrs	1 week	2 weeks	3 weeks	4 weeks
Control	(Y.S.) (1)	6.75f (4)	7.56f	7.23f	7.43k	8.0h	7.88j
RC1b8(2)	Ras cheese	7.43e	8.95c	8.95d	8.93g	8.47fg	8.36i
RC1b8+Y.S.(3)		8.23cd	8.95bc	8.98d	8.72h	8.43fg	8.47hi
RC4b2	Ras cheese	8.30cd	9.23d	9.6c	10.67d	10.6c	8.98g
RC4b2+ Y.S.		8.23cd	8.47d	9.11d	9.47f	8.23gh	7.47k
FC1b1	Domiatii cheese	8.91ab	10.23a	11.23a	11.88a	11.86a	11.43b
FC1b1+ Y.S.		8.75bc	8.96bc	9.82bc	11.11c	11.47b	10.3d
SC1a4	Karish cheese	8.91ab	10.36a	11.23a	11.36b	11.72a	11.63a
SC1a4+ Y.S.		9.0a	9.23b	9.88b	10.86d	10.84c	9.6e
S4b1	Raybe milk	38.0d	10.36a	11.23a	11.84a	11.36b	11.11c
S4b1+ Y.S.		8.36cd	9.11bc	9.86bc	10.79d	10.0e	10.23d
LZ1b8	Zeer milk	8.11d	9.11bc	8.91d	8.75gh	8.51f	8.47hi
LZ1b8+ Y.S.		8.9cd	9.0bc	8.86d	8.51i	8.47fg	8.56h
LZ1a7	Zeer milk	8.23cd	8.91c	9.56c	9.86e	10.3d	9.23f
LZ1a7+ Y.S.		8.11d	8.11e	8.0e	8.11j	7.67i	7.36k

Table 2: Stability of probiotic isolates or in the presence of a traditional yoghurt starter (YS) culture during the storage of yoghurts at 4 ◌̊C for 4 weeks.

(1) Y.S. = Traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus.*

(2) Probiotic isolate alone as a starter (in skim milk). (3) Probiotic isolate mixed with Y.S. at a ratio of 1:1.

(4) Means a column followed by the same letter (s) are not significantly different at 1% level of probability (Duncan's multiple-range test, Duncan, 1955).

In comparison, the isolates alone showed almost a similar pattern, where the counts increased variably till the 3rd week, followed by a considerable decline in the 4th week in most of the isolates. The viable counts for all the isolates alone remained always higher than the control. Besides, the isolates FC1b1 and SC1a4 alone gave the highest counts as compared to the other isolates throughout the whole period of storage. The major differences between the probiotics survival were related to species differences and there was little variance between different commercial strains of the same *Bifidobacterium* or *L. acidophilus* . All *Bifidobacterium sp*.survived well, as did *L. casei, L. paracasei and L. rhamnosus*. *Lactobacillus strains* (L26, Lc1 and DR20) showed survival patterns similar to the *Bifidobacterium spp*.The *L. casei* (Lc1) maintained at the initial levels and then decreased slightly by 32 weeks to be present at 1.6×107 CFU/g(Phillips *et al*,2006).

As for the mixtures (isolates + Y.S.), the viable counts obviously increased in all treatments till the 3rd week, then the counts showed a slight increase or remained stable in certain cases, i.e. S4b1+Y.S., LZ1b8+Y.S. and RC1b8+Y.S., while a considerable decline in those counts occurred in the rest treatments. Also, no inhibition was evident from the two starter cultures against the lactobacilli strains. This allows the co-existence of the two starter cultures with the probiotics isolates in yoghurt (Maragkoudakis *et al*., 2006).

In agreement with the present results, Haddadin *et al*. (2004) produced yoghurts containing counts of > 1.0 x 108 cfu / ml of the individual probiotics and high counts of the traditional species, and storage trials at 4 ◌̊C. showed that the viability of the probiotic cultures was retained over 15 days. It has been suggested that the probiotic

bacterial population in a fermented milk product should be above 6 log cfu g_1 at the end of its shelf life, in order to exert any beneficial effect on the host (Shah *et al*., 1995; Schillinger, 1999and Svensson, 1999). Also, Linn *et al*. (2008) recorded counts of > 1.0 x 109 cfu / ml for the yoghurts based on the individual probiotics, and high counts for the traditional species were also obtained. Storage trials at 4 ◌̊C showed that the viability of the probiotic cultures was retained over 3 weeks then decreased to 2.8 x 106 on the 28th day. These observations were consistent with the findings of Akalin *et al*. (2004)and Dave and Shah (1997). Who reported higher stability of ST than LB and bifidobacteria in probiotic yoghurts during storage time.

In addition, Tabatabaie and Mortazavi (2008) concluded that the survival of the two probiotic strains *L. rhamnosus* and *Bifidobacterium bifidum* was found to be extremely stable during 5 weeks storage period at 4 ◌̊C.The initial counts of ST in all inoculated milks ranged from5.3 to 5.5 log CFU/ml that increased by ca. 3 log cycles after overnight incubation Paseephol, and Sherkat. (2009).

The experimental and control yoghurt s showed ability in sustaining high numbers of ST up to 14 days of storage (P > 0.05) and only a marginal decline occurred in the following 14 days. After 28 days, all yoghurts contained >8.0 log CFU/g of ST,decreasing from the initial counts by only 2.7–4.2%. This reflected the high stability of ST in the products Paseephol.and Sherkat. (2 0 0 9).

C- Firmness of yoghurt gel (curd tension):

Table (3) shows the changes in the firmness of yoghurt gel during refrigerated storage for 4 weeks. In fresh yoghurt, the curd tension (C.T.) was higher for all treatments of the isolates mixed with Y.S. (53.0 – 63.2 gm) than the control 52.4 gm, and the highest value was recorded for LZ1a7+Y.S. treatment. Meanwhile, the treatment of all isolates alone exhibited much lower C.T. values ranged (21.8 – 41.1 gm), and the highest values was recorded for FC1b1 treatment.

Table 3: Curd tension changes of yoghurt based on probiotic isolates alone or mixed with traditional starter during storage at 4 ^{0}C.

Treatment (Starter)	Source	pH of yoghurt stored at 4C.				
		24 hrs	1 week	2 weeks	3 weeks	4 weeks
Control	(Y.S.) (1)	52.4	43.8	32.8	28.3	42.6
RC1b8(2)	Ras cheese	21.8	18.3	12.0	14.4	23.2
RC1b8+Y.S.(3)		57.0	42.0	32.8	40.0	41.5
RC4b2	Ras cheese	33.5	45.6	37.9	63.7	42.4
RC4b2+ Y.S.		63.0	55.8	33.7	58.2	54.9
FC1b1	Domiatii	41.1	43.9	34.1	51.9	45.6
FC1b1+ Y.S.	cheese	56.2	54.6	39.2	63.1	52.3
SC1a4	Karish cheese	28.5	25.6	23.3	36.5	35.0
SC1a4+ Y.S.		60.1	54.7	39.0	58.7	56.4
S4b1	Raybe milk	38.1	43.9	25.7	55.7	49.1
S4b1+ Y.S.		60.8	54.1	36.1	51.3	51.9
LZ1b8	Zeer milk	35.9	47.5	41.9	40.0	44.6
LZ1b8+ Y.S.		53.0	42.0	36.2	35.6	39.6
LZ1a7	Zeer milk	25.4	40.1	27.9	40.4	47.3
LZ1a7+ Y.S.		63.2	52.3	44.9	50.7	61.0

(1) Y.S.= Traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus.*

(2) Probiotic isolate alone as a starter (in skim milk).

(3) Probiotic isolate mixed with Y.S. at a ratio of 1:1.

A noticeable decline then occurred in C.T. in treatments and the control till the 2nd week of storage. In the 4th week, treatments of the isolates LZ1a7, LZ1b8, and their mixture with Y.S., as well as the control exhibited some increase in C.T., while the rest of treatments showed some decline in C.T.

The physical character of the curd is of primary importance with reference to the consistency of cultured yoghurt. In general, the obtained results of fresh yoghurt indicate that the curd tension values coincided greatly with the previous results of lower pH, and vice versa (Table 1). This is in agreement with Mohammad (2009) who concluded that the higher the pH level, the lower the curd tension occurred. It is also necessary to explain the changes in yoghurt during storage, the yoghurt gel is not a tight matrix but formed as a loose structure with fractal characteristics, however, post acidification over the storage period and consequently casein arrangement around the bacterial cells could result in a more compact and continuous structure (Hassan *et al*., 1996).

D- Syneresis:

Table (4) presents the syneresis (ml/ 100gm within 30 minutes) for all yoghurt treatments and the control. The syneresis, in fresh yoghurt, was 26.3 ml for the control as compared to (22.8-35.0 ml) for the treatments of the individual isolates, and (21.0-29.3 ml) for the treatments of isolates +Y.S. Measurement of syneresis fluctuated throughout the whole period of storage till the 4th week, at which time a noticeable decline in syneresis occurred in most treatment.

Table 4: Syneresis of yoghurt based on probiotic isolates alone or mixed with traditional starter (YS) during storage at 4 ◌̊C.

Treatment (Starter)	Source	pH of yoghurt stored at 4C.				
		24 hrs	1 week	2 weeks	3 weeks	4 weeks
Control	(Y.S.) (1)	26.3	30.5	28.0	18.5	20.0
RC1b8(2)	Ras cheese	35.0	31.0	37.5	20.0	24.0
RC1b8+Y.S.(3)		21.8	27.0	31.5	28.4	24.0
RC4b2	Ras cheese	27.3	22.0	27.0	11.0	26.0
RC4b2+ Y.S.		29.3	27.0	27.0	25.0	29.0
FC1b1	Domiatii	26.8	23.0	24.0	21.0	24.0
FC1b1+ Y.S.	cheese	24.8	28.0	31.0	21.0	19.0
SC1a4	Karish cheese	22.8	21.0	23.0	21.0	26.0
SC1a4+ Y.S.		21.0	26.0	31.0	19.0	27.0
S4b1	Raybe milk	32.0	30.5	29.0	19.0	18.0
S4b1+ Y.S.		25.8	22.0	24.0	23.0	20.0
LZ1b8	Zeer milk	23.0	31.5	27.5	27.0	31.0
LZ1b8+ Y.S.		28.5	25.5	28.5	10.0	24.0
LZ1a7	Zeer milk	28.3	34.0	28.0	22.0	19.0
LZ1a7+ Y.S.		26.3	31.0	18.0	27.0	21.0

(1) Syneresis: Amount of whey drained (ml).
(2) Y.S. = Traditional starter contained standard yoghurt culture of *Lactobacillus bulgaricus* + *Streptococcus thermophilus*..
(3) Probiotic isolate alone as a starter (in skim milk).
(4) Probiotic isolate mixed with Y.S. at a ratio of 1:1.

It is well apparent, in the present results, that the ranges of syneresis for both the treatments of the isolates alone or mixed with Y.S. were almost closely

related, and that of the control lies in the middle of these ranges. However, It seems that there was no much relation to the curd tension as previously reported by Mohammad (2009) whose work based only on 3 treatment.

REFERENCES

Akalin A. S., S. Fend,erya and Akbulut N. (2004). Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. Inter. J. Food Sci. & Technol., 39 (6): 613-621.

Ali, F. S., Saad O. A. and Salwa A. H. (2010). The production of probiotic yougurt.I.Isolation and Screening of probiotic bacteri. The 5th Arab Conference for Food Technology and Dairy Sciences. October 18-22. Mansoura/Luxure, Mansoura Univ., Egypt.

Ali, F. S., Saad O. A. and Salwa A. H. (2012). Production of probiotic yoghurt. Minia International Conference for Agriculture and Irrigation in the Nile Basin Countries, El-Minia, Egypt, 26th to 29th March.

Botić, T., Klingberg T. D., Weingartl H. and Cencič A. (2007). A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. International J Food Microbiology 115: 227–234.

Dave, R.I. and Shah N.P. (1997). Viability of yogurt and probiotic bacteriain yoghurts made from commercial starter culture. Int. Dairy J. 7: 31–41.

Donkor, O.N., Henriksson A., Vasiljevic T. and Shah N.P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. Int. Dairy J. 16 (10), 1181–1189.

Duncan, D.B. (1955). Multiple range and multiple F- tests. Biometrics, 11: 1-42.

Haddadin, M.S.Y., Awaisheh S.S. and Robinson R. K. (2004). The production of yoghurt with probiotic bacteria isolated from infants in Jordan. Pakistan J. Nutr., 3 (5): 290-293.

Hassan, A. N.; Frank J. F.; Schmidt K. A. and Shalabi S. I. (1996). Texture properties of yoghurt made with encapsulated non-ropy lactic cultures. J. Dairy Sci., 79:2098– 210.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT 39: 1221–1227.

Kailasapathy, K. and Rybka S. (1997). *Lactobacillus acidophilus* and *Bifidobacterium* spp.: Their therapeutic potential and survival inyogurt. The Australian J. Dairy Technology, 52: 28–35.

Knorr, D. (1998). Technology aspects related to microorganisms infunctional foods. Trends in Food Science Technology, 9: 295–306.

Linn, Z. M., Aye K. N. and Yu S. S. (2008). Processing of Probiotic Yogurt GMSARN Int. Conf. Sustainable Dev.: Issues and Prospects for the GMS, 12-14 Nov., p:1-6.

Lourens-Hattingh, A. and Viljoen B. C. (2001). Review: Yoghurt asprobiotic carrier food. Int J Dairy, 11: 1–17.

Maragkoudakis, P. A.; Zoumpopoulou, G.; Miaris, C.; Kalantzopoulos, G. B. and Tsakalidou, E. (2006). Yoghurt production and analysis. Int Dairy J., 16: 52-60.

Marshal, V.M. and Tamime A.Y. (1997). Starter cultures employed in manufacture of bio-fermented milk. Int. Dairy J., 5: 35 – 39.

Mohammad, A.E.D.A. (2009). Microbiological and technological studies on some starter-like bacteria isolated from naturally fermented milk products. M.S., Dairy Sci.

Dept., Fac. Agric., Minia Univ., 119p.

Paseephol, T. and Sherkat F. (2009). Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. J Functional Foods 1:311-318.

Phillips, M, Kailasapathy K. and Tran L. (2006). Viability of commercial probiotic cultures (*L. acidophilus, Bifidobacteriumsp., L. casei, L. paracasei* and *L. rhamnosus*) in cheddar cheese. Int J Food Microbiology 108: 276–280.

Robinson, R. K. (1987). Survival of *Lactobacillus acidophilus* in fermented products. Suid Afrikaanse Tydkrif Vir Suiwelkunde, 19: 25–27.

Ross, R.P., Fitzgerald G., Collins K. and Stanton C. (2002). Cheese delivering biocultures-probiotic cheese. The Australian J. Dairy Technology 57: 71–78.

Rybaka, S. and Kailasapthy K. (1995). The survival of culture bacteria in fresh and freeze dried yoghurt. Austr. J. Dairy Tech., 50(2): 51-57.

Salminen, S., Deighton M. and Gorbach S. (1993). In S. Salminen & A. Von Wright (Eds.), Lactic acid bacteria (pp. 199-225). Marcel Dekker.

Schillinger, U. (1999). Isolation and identification of lactobacilli from novel-type probiotic and mild yoghurts and their stability during refrigerated storage. Int J Food Microbiology, 47: 79-87.

Shah, N. P., Lankaputhra, W. E. V., Britz, M. L., and Kyle, W. S. A. (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. Int J Dairy, 5: 515–521.

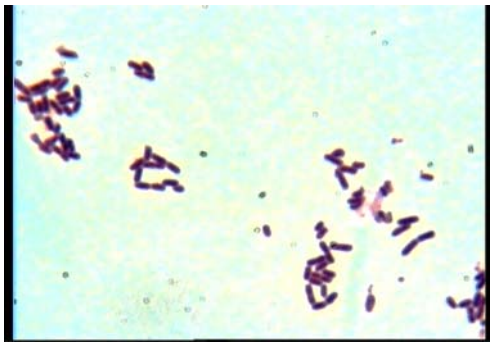
Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science, 83: 894–907.

Svensson, U. (1999). Industrial perspectives. In G. W. Tannock (Ed.), Probiotics, a critical review (pp. 46–57). Norfolk, UK: Horizon Scientific Press.

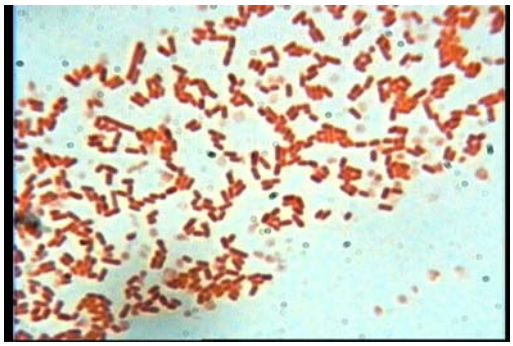
Tabatabaie, F. and Mortazavi A. (2008). Influence of lactulose on the survival of probiotic strains in yoghurt. World Appl. Sci. J., 3 (1): 88-90.

Tamime, A. Y., Saarela M., Sondergaard A. K., Mistry V. V. and Shah, N. P. (2005). Production and maintenance of viability of probiotic micro-organisms in dairy products. In A. Y. Tamime (Ed.), Probiotic dairy products (pp. 39–72). Oxford: Blackwell Publishing.

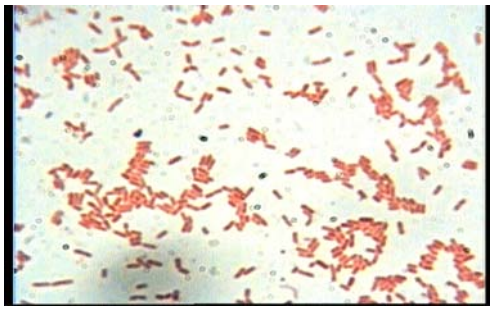
Tannock, G. W., Munro, K., Harmsen, H. J. M., Welling, G. W., Smart, J. and Gopal P. K. (2000). Analysis of the faecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR 20. Applied and Environmental Microbiology, 66: 2578–2588.



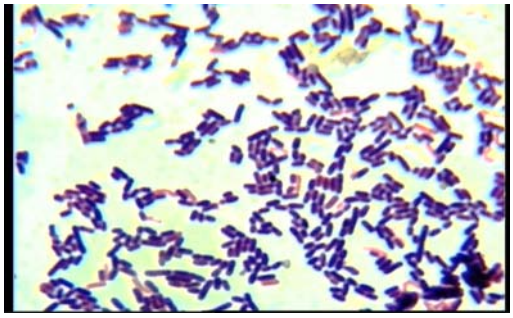
RC1 b8 Ras Cheese (*Bifidobacterium* spp.)



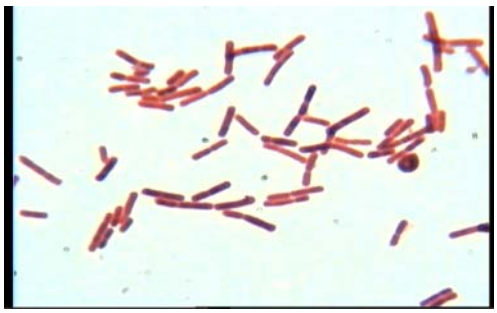
C4b2RasCheese (*Bifidobacterium* spp.)



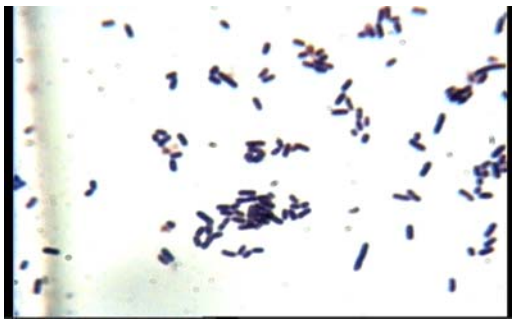
FC1b1 Domiati Chesse (*Bifidobacterium* spp.)



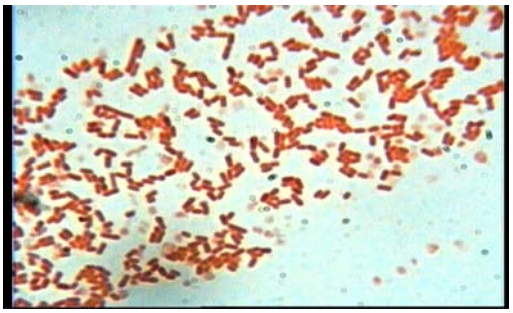
SC1a4 Kariesh Cheese (*Bifidobacterium* spp.)



S4b1 Raib milk (*Lactobacillus* spp.)



LZ1b8 Zeer milk (*Bifidobacterium* spp.)



LZ1a7 Zeer milk (*Bifidobacterium* spp.)

Fig. 1: Bacterial shape of good clotting probiotic isolates.

ARABIC SUMMARY

ثبات بكتريا البروبيوتيك فى الزبادي خلال التخزين فى المبرد

فاروق شحاتة على - عمر عبد اللطيف عمر سعد- سلوى عادل حسين غريب
قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – المنيا- مصر .

سلالات البروبيوتيك المعزولة من الجبنة الرومى والدمياطى والقريش والمنتجات المتخمرة(الرايب ولبن الزير) على بيئة الMRS pH6.3 و تم اختبارها من حيث شدة تكوين الخثرة فى اللبن الفرز.تم تعريف السبع عزلات والتى أدت الى تخثير اللبن:
Bifidobacterium spp. (RC1 b8, RC2 b1, FC1b1, SC1a4, LZ1b8 and LZa7) , والسلالة السابعة *Lactobacillus* spp. (S4b1)
اختيرت اقوى سبع سلالات والمكونة للخثرة فى اللبن الفرز فى انتاج زبادى 100% بروبيوتيك و50% بروبيوتيك مع 50% بكتريا الزبادى العادى مع المقارنة ابضا مع100% بكتريا الزبادى العادى تم دراسة التغير فى الـ pH وثبات اعداد بكتريا البروبيوتيك و الصفات الطبيعية (قوة الخثرة والتشريش) للزبادى المخزن حتى أربع أسابيع على4°م.